to about 50°C. As can be seen from Figure 7 of D1, all of the enzymes disclosed including Ni-TX8 exhibit less than 40% of their optimal activity ranging from about pH 4.2 to about pH 4.5. Ni-TX8 exhibits less than 40% optimal activity at about pH 4.5. There is no teaching that Ni-TX8, or any other modified xylanase exhibits 40% of its optimal activity down to pH 3.5 as required by claim 1. As a result, this disclosure cannot be cited against claim 1 under Article 33(2) PCT. Since claims 2, 6, and 8 to 11 all depend from claim 1, Applicant respectfully submits that the objection under Article 33(2) PCT be removed from these claims.

Claims 23-25 and 27 have been objected to under Article 33(2) PCT in view of D2. It is stated that D2 discloses obtaining a xylanase from *Microtetraspora flexuosa* or *Thermonospora fusca*, that is characterized as being stable after a 1 minute heat shock at 95°C and that is active at pH 6.5 and at 40°C. Applicant respectfully disagrees with the rejection of amended claim 23 and its dependent claims in view of D2 under Article 33(2) PCT.

Claim 23, as now amended, is directed to a method for obtaining a Family 11 xylanase with a set of defined properties. Of the xylanases disclosed in D2, only one exhibits greater than 40% of its optimal activity above the temperature of approximately 45-50° (see Figure 2) that being xylanase No. 5. Table 1 on page 32, defines as xylanase No. 5 as having a molecular weight of 35kD. It is submitted that a xylanase of this molecular weight is not a Family 11 xylanase. Examiner's attention is drawn to page 12 of the present application, last paragraph, lines 23-31 which defines the properties of a Family 11 xylanase. One of the properties of a Family 11 xylanase is that they have molecular weights of approximately 20kD. Therefore, Examiner is respectfully requested to remove the objection to claim 23, and dependant claims 24, 25 and claim 27 under Article 33(2) PCT in view of the present amendment.

Claims 1, 2, 9, 10, 15 and 16 have been objected to under Article 33(2) PCT as lacking novelty in view of D3. D3 discloses several xylanase mutants derived from a *Bacillus circulans* xylanase having disulfide bridges. Applicant respectfully disagrees with the application of D3 against claims 1, 2, 9, 10, 15 and 16.

Examiner's attention is directed to Figure 27 (also see supporting text of Figure 27 in the paragraph spanning pages 162-163) of D3 which shows that below about pH 4.5 to 4.2, less than 40% of the optimal activity of the enzymes is obtained. These enzymes clearly do not exhibit 40% of their optimal activity down to pH3.5 as required by claim 1. Claims 2, 9, 10, 15 and 16 all depend from claim 1, and therefore Applicant respectfully submits that the objection against these claims under Article 33(2) PCT be removed.

INVENTIVE STEP

The claims of the present invention have been objected to under Article 33(3) PCT in view of D1 in combination with D4.

Examiner states that a mutant is disclosed within D1 that comprises a Q162H mutation (NI-TX1), and notes that this mutation has no affect on the thermostability of xylanase. However, Examiner suggests that the combination of D1, disclosing the Q126H mutation, and D4, teaching increased thermostability using disulfide bridges, renders obvious the mutations of the present application. Applicant respectfully disagrees with Examiner assessment in this regard.

The altered amino acid composition of the mutation present within N1-TX1 is defined on page 11, Table 2 of D1. Ni-TX1 comprises a mutation at Q162H along with TvX(3-190). The TvX(3-190) mutation includes 5 further altered residues at positions 1, 2, 4 and 9, 65 and 143. Therefore there is no disclosure of the Q126H mutation, alone, or of a xylanase comprising Q162H that has any effect on the thermostability or alkalanophilic properties associated with this mutation. Furthermore, there is no suggestion within D4 that the mutation at position 162, alone, or in combination with the other disclosed positions could account for increased thermostability and alkalanophilicity as described and claimed in the present application. There is no disclosure within D1, D4, or a combination of D1 and D4, that would suggest one of skill in the art to obtain a xylanase having increased thermostability and alkalanophilicity, in general, or through the use of a Q162H mutation as disclosed and claimed in this application.

In paragraph 3 Examiner suggests that D4 discloses the use of disulfide bridges to increase the thermostability of Family 11 xylanases. Applicant notes that the claims that include the addition of disulfide bridges, those being claims 15 and 16, all each ultimately depend from claim 1. It is submitted that the subject matter of claim 1 is not suggested or disclosed in any of the cited documents including D4, and therefore, that the subject matter of claims 15 and 16 is not obvious in view of D4.

Applicant respectfully submits that the objections to the claims of the present application under Article 33(3) PCT be removed from the present application.

Respectfully submitted

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Enclosures.

CLAIMS

1. An isolated, modified, Family 11 xylanase characterized in exhibiting at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 50°C, said xylanase being thermostable.

2. The isolated xylanase of claim 1 wherein said xylanase is characterized in exhibiting at least 40% of optimal activity from about 40 to about 60°C.

3. The isolated xylanase of claim 2 wherein said thermostability is characterized by said xylanase exhibiting at least 30% of optimal activity after a pre-incubation step for 30 minutes at 70°C in the presence of 40% glycerol.

4. The isolated xylanase of claim 2 wherein said thermostability is characterized by said xylanase exhibiting at least 30% of optimal activity after a pre-incubation step for 30 minutes at 80°C in the presence of 40% glycerol.

5. The isolated xylanase of claim 2 wherein said thermostability is characterized by said xylanase exhibiting at least 30% of optimal activity after a pre-incubation step for 30 minutes at 90°C in the presence of 40% glycerol.

6. The isolated xylanase of claim 2 wherein said thermostability is characterized by said xylanase exhibiting at least 30% of optimal activity after a pre-incubation step for 60 minutes at 62.5°C.

7. The isolated xylanase of claim 5 wherein said thermostability is determined in the absence of stabilizer.

8. The isolated xylanase of claim 6 wherein said thermostability is determined in the absence of stabilizer.

9. The isolated xylanase of claim 2, wherein said xylanase is a modified xylanase.

10. The isolated xylanase of claim 9, wherein said xylanase is a Family 11 xylanase.

11. The isolated xylanase of claim 10, wherein said Family 11 xylanase is a *Trichoderma* xylanase.

12. A modified xylanase comprising a basic amino acid at position 162 (TrX numbering) or its equivalent, exhibiting at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 60°C, said modified xylanase being thermostable.

13. The modified xylanase of claim 12, wherein said basic amino acid is selected from the group consisting of lysine, arginine and histidine.

14. The modified xylanase of claim 13, wherein said basic amino acid is histidine.

15. The modified xylanase of claim 9 comprising at least one disulfide bridge.

16 The modified xylanase of claim 9 comprising two disulfide bridges.

17. The modified xylanase of claim 9 comprising a basic amino acid at position 162 (TrX numbering) or its equivalent position, and at least one disulfide bridge.

18 The modified xylanase of claim 9 selected from the group consisting of TrX-162H-DS1, TrX-162H-DS2, TrX-162H-DS4, and TrX-DS8.

19. The modified xylanase of claim 18, wherein said xylanase is TrX-162H-DS1.

20. The modified xylanase of claim 18, wherein said xylanase is TrX-162H-DS2.

21. The modified xylanase of claim 18, wherein said xylanase is TrX-162H-DS4.

22. The modified xylanase of claim 18, wherein said xylanase is TrX-DS8.

23. A method of obtaining a Family 11 xylanase comprising:

i) selecting an organism that expresses xylanase activity, and obtaining said xylanase from said organism;

ii) determining whether said xylanase exhibits at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 60°C; and

iii) determining whether said xylanase is thermostable, and whether said xylanase is a Family 11 xylanase; and

iv) retaining said xylanase that express these properties.

24. The method of claim 24, wherein step i) includes partially purifying said xylanase.

25. A method of preparing animal feed comprising applying the isolated xylanase of claim 1 onto said animal feed to produce a xylanase-animal feed combination, and heat sterilizing said xylanase-animal feed combination.

26. The method of claim 25, wherein said animal feed is a poultry or swine feed.

27. A method of preparing animal feed comprising, applying the xylanase obtained from step iv) of claim 23 onto said animal feed to produce a xylanase-animal feed combination, and heat sterilizing said xylanase-animal feed combination.

28. The method of claim 27, wherein said animal feed is a poultry or swine feed.

29. An isolated recombinant xylanase characterized in exhibiting at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 50°C, said recombinant xylanase being thermostable.

30. The modified xylanase of claim 12 comprising at least one disulfide bridge.

31. The modified xylanase of claim 12 comprising two disulfide bridges.